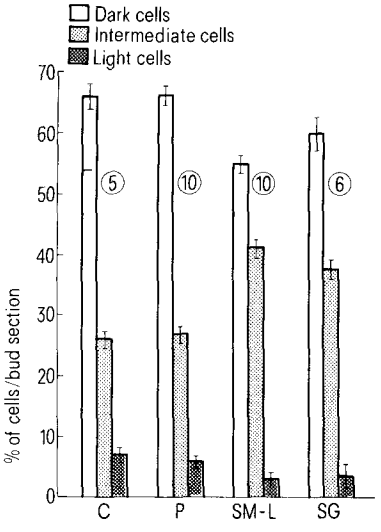
removed and fixed in the Karnowsky mixture[9] buffered with 0.2 M collidine. The material was postfixed in OsO_4, dehydrated and embedded in Epon 812. Serial sections, each 1 µm in thickness, were obtained and stained with toluidine blue-borax (pH 11). Only obliquously sectioned buds were selected for counting of cells. The longitudinal and cross-sectioned buds were not suitable for this purpose.

The in-block removal of parotids, submaxillary and sublingual glands significantly increased the rate of intermediate cells with respect to control preparations (figure). No changes in the rates of dark and light cells were detected.

Bilateral removal of parotid glands did not produce structural changes in the taste buds (figure). Bilateral removal of submaxillary and sublingual glands dealt with an increase in the rate of intermediate cells comparable to that observed after the in-block removal of the salivary glands (figure). Although dark cells did not vary in number with respect to the control, their percentage decreased proportionally to the increase in intermediate cells.

Present results support previous investigations on the effect of sialectomy on differentiation and maturation of taste bud cells in the vallate papilla of the rat[8]. The significant increase of intermediate cell rates in the 15-day sialectomized rats points to a stimulation of bud cell maturation.



Effects of the salivary glands on taste buds cells percentages in the vallate papilla of rat. C control buds; P, animal deprived of parotid glands; Sm-Sl: submaxillary sublingual glands removal; SG: in-block removal of the main salivary glands; Number: number of animal. Mean±SEM.

Taste bud cells have a mean life of approximately 10 days[4], so the absence of a comparable decrease in the number of dark cells was regarded as due to a simultaneous stimulation of cell differentiation. The absence of significant changes in the rate of light cells suggests that the involution of the taste bud cells was not primarily affected by sialectomy. This, however, does not preclude the possibility that changes in this cell stage might be found in longer-term experiments.

The bilateral removal of parotid glands did not affect the life cycle of taste bud cells, but removal of submaxillary and sublingual glands produced changes comparable to those observed after sialectomy. This finding suggests that these salivary glands are involved in the mechanism controlling the life cycle of taste bud cells. Whether the products responsible for such an effect are secreted, concentrated or activated by submaxillary and sublingual glands, cannot be ascertained at present. It has been shown that all of these processes take place in the salivary glands.

The submaxillary glands have 'hormonal functions' through secretion of the nerve growth factor[10], the epithelial growth factor[11], steroids[12] and other products. On the other hand, the submaxillary gland is known to concentrate some circulating hormones[13]. Finally, it has been reported that testosterone[14] and progesterone[15] are transformed into other products in the submaxillary glands. Additional investigation is needed to gain a better understanding of the mechanism by which salivary glands contribute to the regulation of the life cycle of taste bud cells.

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Directed biosynthesis of analogues of ergot peptide alkaloids with *Claviceps purpurea*

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Summary. Analogues of peptide ergot alkaloids can be obtained by feeding a producing culture of *Claviceps purpurea* with an analogue of one of the amino acids of the peptide chain of the alkaloid.

It is known that the biosynthesis of the peptide antibiotics is catalyzed by polyenzyme complexes, and that these enzymes have a rather broad specificity with the consequence that structurally similar amino acids can replace certain of the amino acid residues of the peptide chain during the antibiotic biosynthesis[1].

The ergot alkaloids of the ergotamine and of the ergotoxine groups are formed by a moiety of lysergic acid linked to a

cyclol peptide side chain derived from 3 amino acids. The amino acids present in the chain characterize the different alkaloids. The biosynthesis of the peptide chain in ergot alkaloids is supposed to take place through a polyenzyme complex[2]. In the present study, it is shown that one of the amino acids of the peptide chain of the ergot alkaloids can be replaced by some of its analogues when added to the producing culture.

Claviceps purpurea S40T phe-producing ergocristine in submerged culture and requiring phenylalanine both for growth and production of alkaloids, was utilized in the first part of this study. The composition of the basic culture media utilized is reported in table 1.

The strain S40T phe$^-$ was grown on slants of medium T2 supplemented with 1 g/l of D,L-phenylalanine, at 28 °C for 8 days and then transferred into 300 ml Erlenmeyer flasks containing 50 ml of the inoculum medium TG/S, with 1 g/l of D,L-phenylalanine. The flasks were incubated for 4 days at 24 °C on a rotary shaker operating at 225 rpm with a 5 cm throw. Aliquots of the culture obtained were used to inoculate 300 ml flasks containing 40 ml of the production medium T26 supplemented with 0.5 g/l D,L-phenylalanine. The flasks were incubated as described for the inoculum cultures. After 4 days' incubation, the flasks were additioned with 4 g/l of D,L-p-chlorophenylalanine, and after further 10 days' incubation, the cultures were harvested and pooled. The pooled cultures yielded 700 µg/ml of total peptide alkaloids.

The broth culture was filtered, the filtrate was discarded and the mycelium was suspended in a 5% aqueous solution of tartaric acid. After vigorous shaking and filtration, the solid was twice reextracted. The filtrates were pooled, made alkaline to pH 9 with 20% NaOH and extracted with methylene chloride several times. The organic phase was washed with water, concentrated and precipitated with n-hexane. A sample of the crude base was hydrolyzed with hydrochloric acid and analyzed[3]: from the amount of p-chlorophenylalanine and phenylalanine present in the hydrolyzate it was calculated that in the crude base ergocristine and 5′-debenzyl-5′-p-chlorobenzylergocristine were present in about 1:2 ratio.

The crystals formed (0.5 g) contained a mixture of ergocristine (35%) and of 5′-debenzyl-5′-p-chlorobenzylergocristine (65%). From the phosphate salt the crude base was obtained by alkalization and extraction with CH_2Cl_2 and was recrystallized from acetone. The crystallized product was chromatographed on preparative silica gel plates eluting with a mixture of $CHCl_3$ and isopropanol (93:7 v/v). A fraction enriched with 5′-debenzyl-5′-chlorobenzylergocristine was recovered. By successive crystallizations from benzene, methanol, acetone, 100 mg of pure product (m.p. 198–200 °C) was isolated. The acid hydrolysis of the peptide moiety yielded the amino acids proline and p-chlorophenylalanine in 1:1 ratio. Its alkaline hydrolysis yielded lysergic acid and 3,3 dimethylpyruvic acid. At the MS analysis, it showed its characteristic fragments[4] at m/e: 376 and 378; 278 and 280; 267, 153, 70. IR, NMR, and elemental analysis confirmed the proposed structure for 5′-debenzyl-5′-p-chlorobenzylergocristine.

It can therefore be concluded that *C. purpurea* S40T phe$^-$ can utilize an analogue of one of the amino acids of the peptide chain of ergocristine with formation of the corresponding ergocristine analogue.

In order to see if this ability was limited to the strain and to the amino acid analogue tested, or, on the contrary, it was a common feature of the strains producing alkaloids, other strains and aminoacids, or analogues of aminoacids, were tested. The results obtained are summarized in table 2.

Table 1. Composition of the culture media

Components	T2	TG/S	T26
Sucrose	100	–	200
Glucose	–	100	–
Succinic acid	–	7.5	14
Asparagine	10	–	–
Yeast extract	0.1	0.1	0.1
KH_2PO_4	0.25	0.5	0.5
$MgSO_4 \cdot 7\,H_2O$	0.2	0.3	0.5
KCl	0.12	–	0.12
$Ca(NO_3)_2 \cdot 4\,H_2O$	1	–	–
$FeSO_4 \cdot 7\,H_2O$	0.02	0.007	0.007
$ZnSO_4 \cdot 7\,H_2O$	0.015	0.006	0.006
NH_4OH	to pH 5.5	to pH 5.2	to pH 5.2
Agar	18	–	–
Tap water	to 1000	–	to 1000
Distilled water	–	to 1000	–

Table 2. Aminoacid analogues incorporation observed with various strains of *C. purpurea*

Strain	Main alkaloid produced	Amino acid or analogue added to the medium	Amount of the alkaloid containing the added aminoacid or analogue (% of the total)
S40 phe-	ergocristine	p-chlorophenylalanine	63
S40	ergocristine	p-chlorophenylalanine	35
S40 phe-	ergocristine	p-fluorophenylalanine	66
S40	ergocristine	p-fluorophenylalanine	35
275 FI	ergotamine	p-fluorophenylalanine	30
B21	ergocornine ergocryptine	L-norvaline	59
B21	ergocornine ergocryptine	L-norleucine	23
B21 leu-	ergocornine ergocryptine	L-alfa aminobutyric acid	15
B21 leu-	ergocornine ergocryptine	5,5,5-trifluoroleucine	21
B21 leu-	ergocornine ergocryptine	Beta hydroxy leucine	11

3 strains producing different alkaloids can incorporate into the molecule of the synthesized alkaloids, amino acid analogues replacing the natural by occurring amino acids thus producing the corresponding alkaloid analogues.

It can therefore be concluded that the enzyme which is at the base of this biosynthetic reaction is not strictly specific. With the same strain and the same analogue, the incorporation is higher in the mutant requiring for growth the very amino acid that will be replaced in the alkaloid (table 2). These results indicate that the biosynthesis of the peptide moiety of the ergot alkaloids is, at least for the part here considered, controlled by the relative concentration of amino acids in the internal pool, and point to the possibility of obtaining new and potentially pharmacologically interesting alkaloids.

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Difference in resistance of subunits A and B of *Vibrio cholerae* toxin (choleragen) to treatment with pronase

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Summary. Disc electrophoresis in sodium dodecyl sulphate, performed on choleragen after incubation with pronase, only showed the band corresponding to the B subunit, while the A subunit was lost. When examined in immunodiffusion, the digested choleragen was still able to precipitate with specific antibodies. On the other hand, toxicity was considerably reduced.

In previous papers, we reported that the exotoxin produced by *Vibrio cholerae* (choleragen) maintained its electrophoretic pattern, toxicity and immunological reactivity when incubated in vitro, under non-denaturing conditions, with trypsin, chymotrypsin[1], elastase or papain[2].

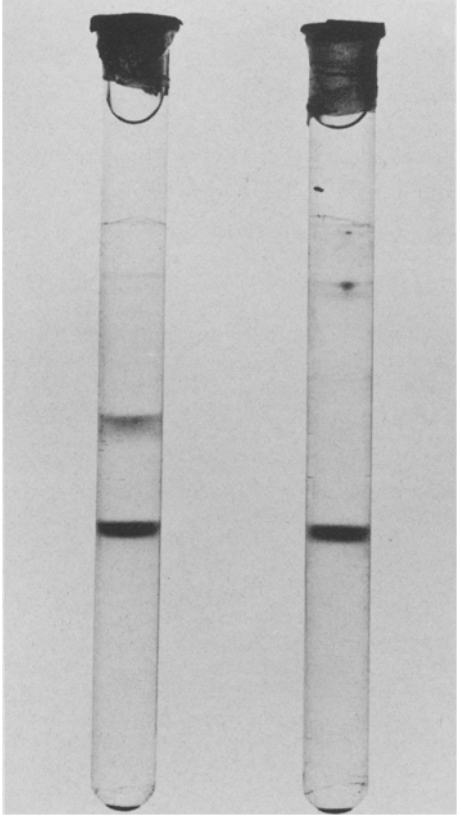

Fig. 1. Sodium dodecylsulphate-polyacrylamide gel electrophoresis of choleragen before (left) and after (right) treatment with pronase.

This remarkable resistance to enzymes showing different types of narrow specificity can be explained on the assumption that choleragen has a native structure in which the peptide bonds, specifically cleaved by the enzymes studied, are not easily accessible.

In order to confirm this hypothesis 'a contrariis', we investigated the effect of incubating choleragen with a mixture of exo- and endo-peptidases with broad specificity, such as pronase, which can attack the protein molecule simultaneously in several different points.

Materials and methods. Highly purified cholera toxin was prepared according to Saletti et al.[3]. The toxin was characterized by physico-chemical, immunological and biological methods: polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS)[4] showed bands corresponding to the A and B subunits, while immunodiffusion on agar plates[5] against a specific antiserum, showed a single band.

The toxic activity, as determined by the skin test in the rabbit[6] was 700,000 blueing doses/mg of protein. Proteins were determined according to Lowry et al.[7]. Pronase Grade B (Calbiochem, USA) was used. The enzymatic activity was determined, according to Narahashi[8], and found to be 690 PU/mg.

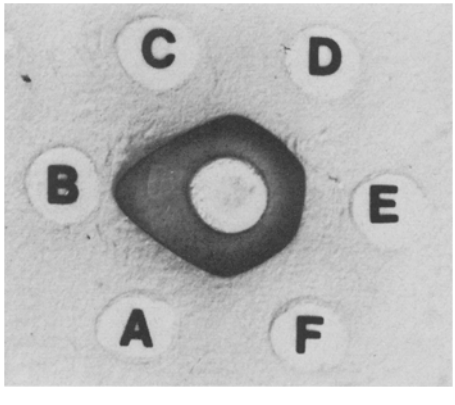

Fig. 2. Agar immunodiffusion of choleragen against the specific antiserum (centre well). A,C,E,F: choleragen; B: pronase; D: choleragen after treatment with pronase.